Pathfinding by the corpus callosum: a role for glial contact guidance in the development of higher brain functions.

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Introduction

Studies of invertebrates have shed light on general principles of axonal pathfinding across phyla (Palka, 1986), but due to the greater complexity and flexibility of mammalian brains, virtually nothing is known about the mechanisms of pathfinding that specifically contribute to what Cook (1986) calls "the brain code" -- the circuitry of neocortical information transfer. A promising heuristic paradigm for such a study is Edelman's (1987) neural Darwinism, which suggests an epigenetic somatic natural selection of "roughly-tuned" neuronal groups as the primary repertoire, further refined after birth (secondary repertoire) by selection using a different mechanism. He proposes that the dominant mechanism for selection of the primary repertoire is by the transient expression of a small set of glycoprotein cell adhesion molecules (CAMs) and substrate adhesion molecules (SAMs). Until recently, however, support for this mechanism has been found only in non-neural, peripheral, or neurula-stage tissues, not in neocortical axonal pathfinding. In contrast, Cook (1986) argues that the corpus callosum is the most revealing place to look for the brain code. With these considerations, then, the corpus callosum appears to be a good proving ground for the significance of proposed mechanisms such as Edelman's in establishing the initial connections of the brain code.

This paper reviews evidence for the centrality of glial cells in the development of the corpus callosum primary repertoire -- that is, innervation of the cortex . Recent experiments that suggest such roles for glial cells will be discussed in relation to

present knowledge about the morphogenesis of the corpus callosum and about likely epigenetic mechanisms.

Morphogenesis of the Primary Repertoire

Invasion of the septum: Pioneering cortical pyramidal axons from both of the embryonic telencephalic vesicles cross through the rostral part of the septum as a tract which then grows caudally as the other fibres are added and the cortex expands (Silver et al., 1982).

Innervation of the cortex: Afferent callosal axons accumulate diffusely below the grey matter for some time before entering and taking up their restricted adult positions. Local conditions determine whether or not they will penetrate, and those that do penetrate do so in the tangentally restricted adult pattern. Most of these are retained, while all others are eliminated (for reviews see Innocenti, 1981, 1988). Cook (1986) discusses models of callosal function that derive from a correspondence between innervating axons and cortical macrocolumns, but recent observations by Swindale (Note 1) throw the whole concept of a modular cortex into disrepute, despite its intrinsic appeal. Nevertheless, a periodic tangental innervation pattern of the corpus callosum in adult cat area 17 was found recently by Hasan (Note 2), which shows an interhemispheric functional correspondence similar to that found in the patches formed by intracortical association fibres within area 18 (Cynader et al., 1987). A similar pattern exists in primate prefrontal association cortex, alternating with intrahemispheric afferents (Goldman-Rakic and Shwartz, 1982).

Differentiation and axon degeneration: Substantial axon elimination is a major determinant of the final neural network of the corpus callosum , but unlike most tissues this does not involve cell death (Windrem, 1988; Innocenti, 1981; Ivy & Killackey, 1982). Axon termination in all layers is synchronous with fast synapse proliferation followed by myelenation, and finally synchronous axon elimination, suggesting neurotrophism and a hormonal trigger (Innocenti, 1988). The axon loss is due entirely to callosal neurons losing their one axon collateral that goes to the contralateral side, while retaining or initiating permanent ipsilateral connections (Ivy & Killackey, 1982). Cortical innervation and collateral loss occur prior to the reorganization of the pyramidal neuron cell bodies into their tangentally restricted adult pattern, suggesting that the final distribution of the callosal neurons is determined by their axons contralaterally (Innocenti, 1981). Only after all of this do the small cortical interneurons differentiate fully.

Likely epigenetic mechanisms

Though cooperative activity of different mechanisms may be essential throughout development of the nervous system (Millaruelo, 1988), most empirical evidence at this time points to adhesion molecules used to label pathways and produce differential specificity. The generality of most other mechanisms is questionable: candidates for chemotaxis are restricted to rare effects of nerve growth factor (NGF) and to negative cases of directional blockage (Gilbert, 1988); the hypothesis of synaptic specificity by chemoaffinity gradients or neuron-specific antigens

lacks direct proof (Gilbert, 1988); neurotrophic effects of NGF and brain-derived neurotrophic factor (BDNF) in vivo are now known, but only in neural-crest derived tissue (Davies, 1988; Hofer & Barde, 1988); and finally, transient neurotransmitter expression in neocortex has been found only in neurons that are themselves transient (Parnavelas & Cavenagh, 1988). Though neurotrophism may play a major role after synaptic contact, it does not seem to be responsible for the initial restriction of axon terminations, which is determined in the waiting period prior to innervation of the grey matter. (Besides, callosal axon elimination occurs postnatally [Innocenti, 1988] during secondary repertoire formation.) On the other hand, examples of contact and cell-adhesion mechanisms are accumulating rapidly (for reviews see Gilbert, 1988; Jacobson, 1988; Posten et al., 1988; Edelman, 1987) and are now known to correlate strongly with pathfinding by the corpus callosum, as follows.

The glial sling

In 1982 Silver et al. showed that a transient glial structure directs the growth of axons across the longitudinal fissure to form the corpus callosum in mice. These glial cells first migrate bilaterally into the dorsal septum and fuse, producing a sling upon which the pioneering callosal axons then migrate. The sling is necessary for corpus callosum pathfinding, and though adult axons maintain the ability to migrate over it, the sling can only be produced by immature glia (Silver et al, 1986).

Anti-GFAP (glial fibrillary acidic protein) staining (Silver et al, 1985) shows a subpopulation of radial glia in the sling,

which form a scaffold. Radial glia have long been known to guide neuroblast migration in cortex (Rakic, 1971), but this was the first indication of their role in axon pathfinding, and it was suggestive that these glia may also have a role in establishing the innervation pattern of afferent axons to the neocortex.

Cortical glia and glycoproteins

In layer IV of rodent primary somatosensory cortex, the topographic representation of the vibrissa fields becomes visible tangentally by Nissl stain as a matrix of cell-sparse "barrels" surrounded by denser "walls" (Wise & Jones, 1978). Each barrel represents one thalamic projection. In 1986, Cooper and Steindler showed that staining with peanut agglutinin (PNA) revealed transient "invisible boundaries" that forecast the later appearance of the Nissl-stained barrels. Marking with anti-GFAP matches the PNA staining pattern, and electron microscopy shows that the cells in these areas look like radial glia (Cooper and Steindler, 1986b). Although this correlation is found for thalamic innervation rather than callosal, and although the staining could be associated solely with extracellular matrix, these correlations are strongly suggestive that the same transient scaffolding of radial glia may be involved in the directing of both initial morphogenesis and of final axon terminations of the corpus callosum.

Subsequent staining correlations in mice with the addition of radiolabeled fucose incorporation (Steindler & Cooper, 1987), reveal transient "hidden boundaries" within and around diencephalon, midbrain, and brainstem nuclei as well as among barrels, all associated closely with glycoproteins, indicating a

"general glial and glycan-related pattern formation principle". The fucose incorporation data imply that these glia synthesize and secrete glycoproteins -- and not glycolipids or glycosaminoglycans -- which confer the key adhesive or recognition properties. Pattern formation in these cases appears to be guided by lamellar expansions of radial glia and maturing astrocytes, which bear a resemblance to the guiding process in cortical layers (Rakic, 1971). Steindler and Cooper suggest the possibility of two different waves of glial glycoprotein expression by immature versus mature astrocytes, manifested by a changing glycan code, and producing first a flexible pattern formation, and secondly a stabilization.

Most recently, one of the results of a study by Godfraind et al (1988) implies that the pattern-formation mechanism outlined above does indeed apply to the corpus callosum during primary-repertoire formation. They showed that the glycoprotein J1 accumulates transiently in the ~~ventricular zone~~ subcortical plate at the time of the early arrival of callosal axons in the mouse embryo. J1 is a neuron-glia adhesion molecule associated with and secreted by glia cells (Kruse et al., 1985).

Conclusion

Permissive or Instructive? A key experiment to determine whether these glia have an instructive or a permissive effect on pathfinding would be to remove a single vibrissa at an early stage and examine the resulting staining patterns. It is known that removing the thalamic vibrissa input prevents appearance of the barrel, and also of the corresponding corpus callosal afferent

(Wise and Jones, 1971). Unfortunately, however, the callosal innervation occurs outside the area containing the barrel fields, so implications for the corpus callosum may not be direct. If glia turn out to be highly instructive, then we have managed to push the question back one stage and we are back where we started asking what causes the pattern formation.

Promise ahead. Whether permissive or instructive, it is promising that the findings of Steindler and Cooper and of Godfraind and colleagues fits so precisely with Edelman's model. It is promising because a new paradigm such as Edelman's is needed in both of the two most mysterious fields in science, both data-rich and theory-poor, poised on the brink of that quantum leap towards cracking the epigenetic and brain codes.

quantum = smallest discrete difference in state, like an electron in different orbitals. The quanta are small.

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Reference Notes

1. Swindale, N.V. 1988. *Is the cerebral cortex a modular structure?* Unpublished manuscript. University of British Columbia.

2. ~~Hasan, S.~~ Matsubara. 1988. Manuscript in preparation. University of British Columbia.